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Lattice Gas Model for Budding Yeast: A New Approach for Density Effects

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Abstract

Yeasts in culture media grow exponentially in early period but eventually stop growing. The saturation of population growth is due to “density effect”. The budding yeast, Saccharomyces cerevisiae, is known to exhibit an age-dependent cell division. Daughter cell, which gives no birth, has longer generation time than mother, because daughter needs maturing period. So far, investigations in exponential growth period have been intensively accumulated, but very little is known for the stage dependence of density effect. Here we present an "in vivo" study of density effect, applying a lattice gas model to explore the age-structure dynamics. It is, however hard to solve basic equations, because they have an infinite number of variables and parameters. The basic equations are constructed from several simplified models which have few variables and parameters. These simplified models are compared with experimental data to report two findings for stage-dependent density effect: 1) paradox of decline birthrate (PDB), and 2) mass suicide of aged mothers. These events suddenly and temporarily occur at early stage of density effect. The mother-daughter model leads to PDB. Namely, when the birthrate of population is decreased, then the fraction of daughter is abruptly increased. Moreover, we find the average age of yeast population suddenly decreases at the inflection point. This means the mass apoptosis of aged mothers. Our results for density effect imply the existence of several types of "pheromones" that specifically inhibit the population growth.

1 Introduction

All biological cells, except for cancer cells, have the density effect: the reproduction (cell division) is disturbed, when cells are crowded. Although the density effect is an important phenomenon in

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microbiology, its investigations are very few. This is because "in vivo" studies are very hard for density effect. Recently, Hagiwara et al (2011) have presented a new experimental technique for the in vivo study. By applying this technique, many findings will be expected for density effect of budding yeasts. Despite they applied lattice gas model, they never fully described the model and the derivation of basic equations. In the present paper, we fully explain the lattice gas model, and report two findings for age-dependent density effect: paradox of decline birthrate (PDB) and mass bursting of aged mothers. Both events occur at the inflection point of logistic curve.

The basic equations have infinite number of variables and parameters, so that it is very hard to solve them. We obtain more simplified models; namely single-cell and mother-daughter models. These are easily solved. On the basis of mother-daughter model, we find PDB is caused by stopping the reproduction of daughter cells. Moreover, we compare an average age $< a >$ of yeast population between theories and experiments. Experimental data indicate the sudden decrease of $< a >$ at the inflection point. This corresponds to the mass deaths of aged mothers.

Experimentally, budding yeast grows by either liquid culture (3-dimensionalal medium) or a plate culture (2-dimensionalal medium). We pay attention only to the liquid culture. The dynamics of yeast growth in a shaking culture is represented by logistic curve. The total population size of yeast initially grows exponentially, but eventually reaches a certain population size (carrying capacity). The saturation of population growth is due to "density effect." In the budding yeast, mothers have a bud scar after budding, while the daughter has no scar (Fig. 1). The daughter has no experience to give birth.

The age dependence of cell cycle in exponential period has been extensively studied by many authors (Carter and Jagadish 1978; Wheals and Lord 1980). Hartwell and Unger (1977) well explained the generation times of both daughter and mother by unequal division model as illustrated in Fig. 1. Here, the blue cell denotes a new born cell which is smaller than mother and called immature daughter (D_i). The immature daughter becomes large and reach the critical size (mature daughter: D_m). The large daughter can reproduce; after giving birth, it becomes mother. The green cells mean large cells which contain both mature daughter (D_m) and mother (M). Thus the generation time of daughter is longer than that of mother, because daughter needs maturity (growth) time to reach a critical size. Namely, $r_d < r_m$, where r_d and r_m are the reproduction rates of daughter and mother, respectively. The HU model has an assumption that the mature daughter is assumed to be identical with mother ("HU assumption"). At present stage, the HU model perfectly is valid in exponential period. In other words, the HU assumption holds without density effect.

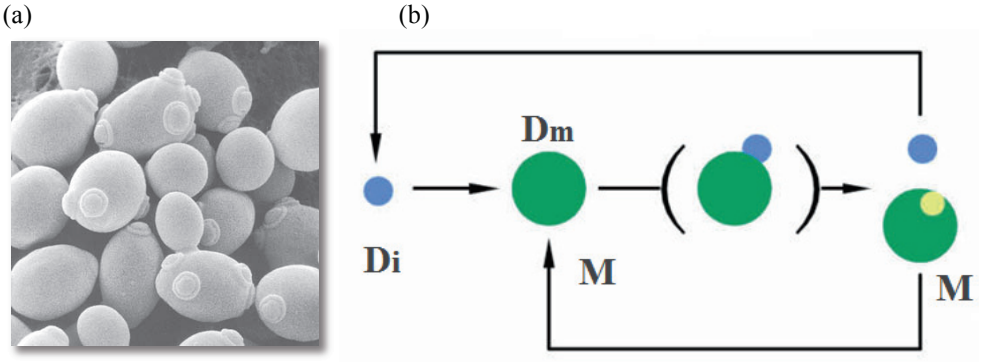

Figure 1: Yeast cells and HU model. (a) Photograph of yeast population, where a wart (crater) denotes bud scar. The age of a cell is defined by the number of scars. (b) The model presented by Hartwell and Unger (1977). The blue cell denotes a new born cell which is smaller than mother and called immature daughter (Di). The immature daughter becomes large and reach the critical size (mature daughter: Dm). Yeast gives birth by

budding. After giving birth, it becomes mother (M). The green color means large cells which is either mature daughter (Dm) or mother (M).

We define "age" of a yeast cell by the total number of bud scars. This is called "reproductive age", and very popular definition for yeasts. Hamada et al (1982) presented the stage-structured dynamics (Hamada model), where each cell is classified by age a which has a scars ($a = 0,1,2,\cdots$). They compared theoretical results with experimental data of the frequencies of age n. The agreement between the theoretical prediction and experimental data in exponential period is fairly good, provided that the HU assumption is applied. This means that the Hamada model with HU assumption works well in exponential period. In contrast, Tainaka et al (2006) have illustrated the invalidity of the HU assumption in the saturation period. They showed the difference between daughter and mothers. When population density becomes considerably high, daughters fail reproduction more easily than mothers. However, they ignore the death (bursting) of cells. Our model presented here contains not only the death but also age structure of yeasts.

2 Models

We apply lattice gas model which is convenience to deal with the density effect (Iwata et al. 2011). So far, various models have been presented for budding yeast. The simplest example is a single cell model, where all yeast cells are regarded to be identical.

1) Single cell model

Each lattice site takes one of two states: empty (O) or yeast cell (X). Fig. 2 is a schematic depiction of lattice gas model. Birth process can be represented by the following chemical reaction:

$$\mathrm{X} + \mathrm{O} \rightarrow 2\mathrm{X} \qquad \text{(rate } r\text{)} \qquad (1)$$

where the parameter r is the reproduction rate. Here we neglect the death process of yeast.

We carry out computer simulation as follows. We randomly and independently choose a pair of lattice sites. If the pair is X and O, then the latter site is changed into X with a rate r. Hence, all sites will be occupied by X, when $t \rightarrow \infty$. The number of total lattice sites denotes the carrying capacity.

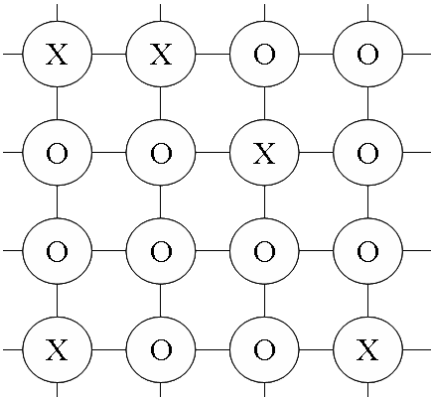

Figure 2: Lattice gas model for single cell model. Each lattice site is either yeast cell (X) or empty (O).

2) Mother-daughter model

The second example is mother-daughter model. Mother and daughter are easily distinguishable by microscope: daughter has no bud scar on the cell surface, while mother has at least one bud scar. Each lattice site therefore takes one of three states: empty (O), daughter (D) and mother (M). Birth process can be represented by the reproduction of either daughter or mother:

$$\mathrm{D} + \mathrm{O} \rightarrow \mathrm{M} + \mathrm{D} \qquad (\text{rate } r_d) \qquad (2a)$$

$$\mathrm{M} + \mathrm{O} \rightarrow \mathrm{M} + \mathrm{D} \qquad (\text{rate } r_m) \qquad (2b)$$

The parameters r_d and r_m are reproduction rates of daughter and mother, respectively. The simulations are performed by the same way as described for the first model (1).

3) General age structure model

The most detailed model has been presented by Hagiwara et al. (2011). This contains all models described in this section. It is powerful to analyze age-structure dynamics. In this model, daughters are separated by either mature (D_m) or immature (D_i) daughters. Furthermore, mothers are classified by their ages. We define M_a be the mother of age a, where a is a natural number ($a = 1,2,\cdots$). We have the following reactions:

$$\mathrm{D_i} \rightarrow \mathrm{D_m} \qquad (\text{rate } g) \qquad (3a)$$

$$\mathrm{D_m} + \mathrm{O} \rightarrow \mathrm{M_1} + \mathrm{D_i} \qquad (\text{rate } r_{md}) \qquad (3b)$$

$$\mathrm{M}_a + \mathrm{O} \rightarrow \mathrm{M}_{a+1} + \mathrm{D_i} \qquad (\text{rate } r_a) \qquad (3c)$$

$$\mathrm{M}_a \rightarrow \mathrm{O} \qquad (\text{rate } d_a) \qquad (3d)$$

The last reaction (3d) means the death process of mothers. The parameters r_{md} and r_a are reproduction rates of mature daughter and mother of age a, respectively ($a = 1,2,\cdots$); d_a is the death rate of age a. We neglect the death process of daughters, according to observations. The death processes (3d) rarely occur, but they cannot be neglected in this model. Because death processes of aged mothers frequently occur for a short while (see Fig. 4).

The simulations are performed as follows. (i) Two-body reactions. We randomly and independently choose a pair of lattice sites, and perform the reactions (3b) and (3c). For instance, if the pair is D_m and O, then the former (latter) site is changed into M_1 (D_i) with the rate r_{md}. (ii) One-body reactions. We randomly choose a lattice sites, and perform the reactions (3a) and (3d). For example, if the site is D_i, it becomes D_m with a rate g. The age-structure model (3) is most powerful. However, it is not convenient, because it contains an infinite number of parameters. For this reason, both models (1) and (2) are useful to solve problems for density effect. It should be noted that all models (1) – (3) are consistent with each other, so long as the death processes are neglected ($d_a = 0$).

3 Theory

3.1 Equations for population dynamics

The lattice gas model has a merit that the population dynamics can be represented by differential equations. Although basic equations are different depending on models, all models are consistent and valid for yeast populations.

1) Single cell model

We first consider the single cell model (1). Let the density of X be x, where the density is equal to the population sizes divided by the total lattice sites. Then the empty density (e) satisfies $e = 1 - x$. The rate equation leads to

$$dx/dt = rx(1-x) \tag{4}$$

where $(1-x)$ in the right hand side means the density of empty sites ($e = 1-x$). This is called logistic equation, and its solution is given by

$$x(t) = \frac{x_0 e^{rt}}{1 + x_0(e^{rt} - 1)} \tag{5}$$

where x_0 is the initial density. When $t \to \infty$, all lattice sites are occupied by yeast cells ($x \to 1$).The population size reaches the number of total lattice sites (carrying capacity). The inflection point is defined by the time satisfying $d^2x/dt^2 = 0$ or $x = 1/2$. In the log-phase, the density is not high ($e \approx 1$), so that we have $x(t) = x_0 e^{rt}$.

2) Mother-daughter model

The densities of daughter (*D*) and mother (*M*) are given by

$$dM/dt = r_d De \tag{6a}$$

$$dD/dt = r_m Me \tag{6b}$$

where the density of empty sites is given by $e = 1 - M - D$. The total density and the fraction of daughter are respectively given by

$$x = M + D \ , \ f_d = D/x, \tag{7}$$

3) General age structure model: Basic equations

The density of empty sites becomes

$$e = 1 - D_i - D_m - \sum_a M_a \tag{8}$$

The time-dependence of immature daughter is given by

$$dD_i/dt = -gD_i + r_{md} D_m e + r_a (\sum_a M_a) e \tag{9a}$$

where the first term in the right hand side has the same meaning in eqn. (7b). The second and third terms correspond to the reproduction of daughter and mothers, respectively. Similarly, we have

$$dD_m/dt = gD_i - r_{md} D_m e$$

(9b)

$$dM_1/dt = r_{md}D_m e - r_m M_1 e - d_1 M_1 \qquad (9c)$$

$$dM_a/dt = r_{a-1}M_{a-1}e - r_a M_a e - d_a M_a \qquad (a=2,3,4,\cdots) \qquad (9d)$$

It is not so easy to solve eqn. (9), because it contains an infinity number of variables and parameters. On the other hand, eqn. (6) is sometimes useful to get some information concerning to mother and daughter. It should be noted that both models (1) and (2) neglect the death process (cell bursting) which occurs for aged mothers. We must take into account the cell death, if we deal with the densities of old ages or the average age of population.

3.2 Determination of parameters

All models are consistent, so long as the death process is neglected. We derive mutual relations between parameters. In log-phase, the cell death can be neglected, and the HU assumption fully agrees with experimental data (Hamada et al. 1982). For this reason, all parameter values are determined by observations in log-phase. The dynamics in log-phase can be characterized by two fundamental quantities: reproduction rate (r) and steady-state fraction of daughter cell (f_d). If the log-phase lasts for a considerably long period, the fraction of daughter becomes constant (steady state). Note that the population size of each age exponentially diverges with the exponent r (e.g. $D \propto e^{rt}$).

In the case of mother-daughter model, the dynamics (6) in log-phase ($e \approx 1$) are given by

$$dM/dt = r_d D, \quad dD/dt = r_m M \qquad (10)$$

If the log-phase lasts for a long period, the fraction of daughter (f_d) becomes constant. Note that the population size of daughter (or mother) exponentially diverges with the exponent r. Inserting

$$D = f_d x = f_d x_0 e^{rt}. \qquad M = (1-f_d)x = (1-f_d)x_0 e^{rt}$$

into eqn. (6), we get

$$r_d = (1-f_d)r/f_d, \qquad r_m = f_d r/(1-f_d) \qquad (11)$$

Hence, all parameters in mother-daughter model can be determined by r and f_d.

Similarly, for basic equations (general age structure model), we have the following relations:

$$g = r(1-f_d)/(2f_d - 1) \qquad (12a)$$

$$r_{md} = r_a = rf_d/(1-f_d) \qquad (12b)$$

$$d_a = 0 \qquad (12c)$$

The last eqn. (12c) is obtained by experimental results (log-phase). The cells never die in log-phase. Hence, all parameters are determined by both r and f_d which are fundamental quantities in log-phase.

3.3 Average age

If we assume $d_a = 0$, then we easily obtain the average age $< a >$ of yeast population. The average age is defined by (the total number of bud scars)/ (the total cell number):

$$< a > = \sum_a (aM_a) \ /[(D_i + D_m) + \sum_a (M_a)] \qquad (13)$$

Note that the factor $(D_i + D_m)$ means the number of daughter. From basic eqn. (9), we have

$$\frac{d}{dt}\sum_a (aM_a) - \frac{d}{dt}[(D_i + D_m) + \sum_a (M_a)] = 0 \qquad (14)$$

Here we assume $M_a \to 0$ for large value of *a*. The eqn. (14) denotes the conservation rule. The biological meaning of conservation rule is very simple: when one cell is born, then one bud scar is increased. So long as the liquid culture last for a considerably long period, the deference in eqn. (14) at initial condition can be neglected. Hence we have

$$< a > = 1 \qquad (15)$$

This holds in log-phase. However, under the density effect, eqn. (15) does not hold as described later.

4 Results

Observed results of age structure dynamics are listed in Table 1, It is found from this table that the number of cells older than 6 is extremely few. This is different from the previous data. Kaeberlein et al. (2005) obtained the life span of the same strain. According to their work, cells survive up to the age 30-40. The discrepancy of life span may come from the different experiments. The experiment of Hagiwara et al. is performed in liquid culture, whereas the experiment of Kaeberlein et al. is performed in plate culture. In the latter case, every daughter is immediately removed (no density effect). For this reason, the life span is extremely shortened by the density effect.

AGE (*a*)	18 hr	21 hr	24 hr	27 hr	30 hr	36 hr
0	58.8	56.8	58.2	59.2	63.6	68.0
1	17.8	19.2	18.2	16.8	14.0	14.4
2	11.8	10.4	8.6	8.6	8.6	6.2
3	5.6	6.6	7.8	7.0	6.2	5.4
4	3.0	3.8	3.2	4.4	3.0	3.0
5	2.2	2.4	2.8	1.8	2.8	1.4
6	0.4	0.6	0.8	1.6	1.0	0.8
7	0.2	0.2	0.2	0.6	0.6	0.6
8	0.2	0.0	0.0	0.0	0.2	0.2
9	0.0	0.0	0.2	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0

Table 1: Dynamics of age structure. Fractions (%) of each age are listed.

The results of mother-daughter model are shown in Fig. 3, where (a) represents the total cell number, and (b) is the fraction of daughter cell (f_d). Here the plots means the experimental data, and the curves are the theoretical predictions. Observations indicate "paradox of decline birthrate" (PDB): when the birthrate of population is decreased, the fraction of daughter is abruptly increased. From

both data of r and f_d in log-phase ($10 < t < 18$), we determine both parameters r_d and r_m by eqn. (11). Note the value of r denotes the slope of straight line in the log-phase. The blue curve is assumed that both parameters are constant. The blue curve never explains PDB. On the other hand, the red curve assumes that the reproduction rate (r_d) of daughter suddenly changes to zero at t =18. This timing denotes the inflection point. The red curve well agrees with experiments (PDB). In other word, the rate r_d should be changed to zero by the density effect.

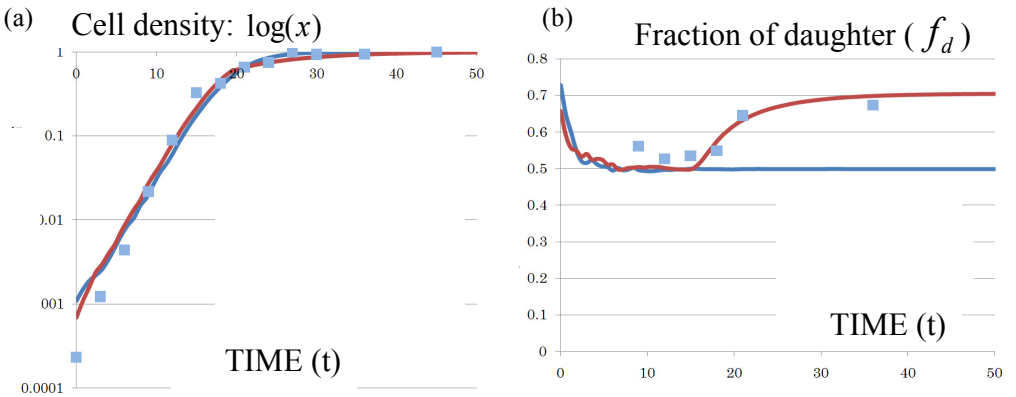

Figure 3: The comparison between theories and experiments. (a) The total cell number and (b) the fraction of daughter cell (f_d). The plots means the experimental data, while the curves are the predictions of mother-daughter model. The blue curve is obtained by eqn. (11) under the assumption that all parameters are constant. The red curve assumes that the reproduction rate (r_d) of daughter suddenly changes to zero for $t \geq 18$.

From Table 1, we can obtain the average age $< a >$ of yeast population (Fig. 4). In Fig. 4 (a), the time dependence of average age $< a >$ in yeast population is depicted. The plots means the experimental data, while the curve is the predictions obtained from general age structure model. Here we ignore the death processes ($d_a = 0$). The experimental results in Fig. 4 (a) suggest that mass bursting of aged mother occur near the inflection point. Because, the average age is suddenly decreased at $t \approx 18$. For this reason, Hagiwara et al. (2011) explored the shapes of cells. They found many wreckages of bursting at the inflection point [see Fig. 4 (b)]. Such wreckages were found only at the inflection point.

(a) Average Age $< a >$ (b) Bursting of Aged Mothers

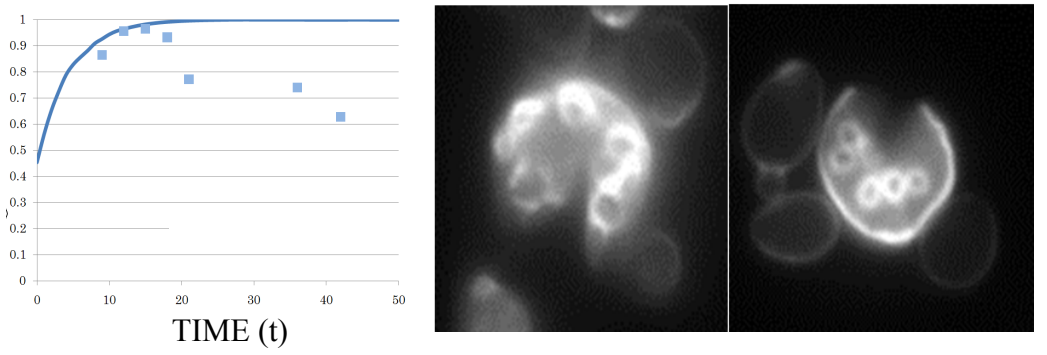

Figure 4: Cell bursting. (a) Time dependence of average age $< a >$ for yeast population is shown. The plots means the experimental data obtained from Table 1, while the curve is the theoretical predictions obtained from general age structure model under the assumption $d_a = 0$. (b) Death (bursting) of aged mothers. The death process simultaneously occurs near inflection point (Hagiwara et al. 2011).

In Fig. 5, the best fitted curve is depicted by assuming $d_a =$ constant for $a \geq 5$. Here (a) and (b) respectively display the average age $< a >$ and the probability of bursting cells among total cells. The agreement between theory and experiment if fairly good. Hence, we can find the wreckages of bursting only at the inflection point. This completely agrees with observations. Note that the initial condition in Fig. 5(a) is not so important, because $< a > \rightarrow 0$ in log-phase [see eqn. (15)].

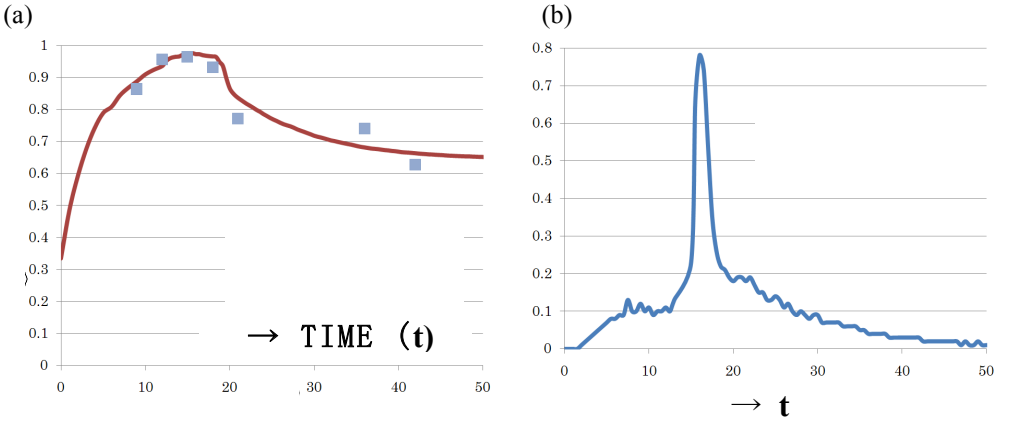

Figure 5: Results of general age structure model. (a) The best fitted curve for average age $< a >$. (b) The probability of bursting cells among total cells.

Conclusion and Discussion

The age structure dynamically changes by density effect. We report two findings: 1) paradox of decline birthrate (PDB), and 2) mass bursting of aged mothers. The bursting of aged mothers can be easily predicted by the average age $< a >$ of yeast population, because $< a >$ is suddenly decreased at the inflection point [see Fig. 4(a)]. The age-structure theory plays an important role for the discovery

of cell bursting: according to the theoretical result of Fig. 5 (b), we get the photographs in Fig. 4(b). Hence, this is rare case that theory leads to new discovery.

The experimental data of PDB is observed in Fig. 3(b). When $t > 18$, the fraction of daughter increases in spite of the decrease of birthrate. The cause of PDB is uniquely found by parameter fitting: as shown in Fig. 3(b), the birthrate of daughter becomes suddenly zero. This cause is easily predicted from the mother-daughter model (2). The reaction (2a) means that daughters give birth; in this case only the number of mother increases. Similarly, the reaction (2b) denote the birth process of mother, and it only increases the number of daughter. What happens by the density effect? wIn other words, which reaction is disturbed (2a) or (2b) after the inflection point ($t > 18$)? The answer is clear by POB: only the birth process of daughter (2a) is disturbed. Both events PDB and mass bursting simultaneously occur at the inflection point. This can be interpreted as a kind of altruism: when daughters stop reproduction, then aged mothers commit suicide.

Several assumptions in our theory are discussed. First, we neglect the population size of old ages ($M_a \to 0$ for large value of a). This assumption is adequate by both experiment (Table 1) and theory. According to the theory of Hamada et al. (1982), stationary structure in log-phase have been analytically obtained as:

$$M_a = (f_d)^{a+1} / q \ , \tag{15}$$

where $q = r_m / r_d$. This power series converge $M_a \to 0$ immediately. Second, we assume the hypothesis that parameter values in log-phase hold even in density-effect phase except for r_d and d_a. Such a hypothesis is plausible. For example, our theory predicts that all daughter cells become large at the final equilibrium; this outcome is confirmed by experiments.

We have developed the in vivo study for density effect of budding yeasts. Yeast is closely related to cancer (Hartwell 2004). Our results imply the existence of several types of "pheromones" that specifically inhibit the population growth. When we use mutants of yeast, various types of density effect are expected (Hagiwara 2012); an example is the bursting of daughter cells. If some causal factors (pheromones) of density effect are detected, then they are useful for cancer study.

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